# Targeting the DNA repair enzyme APE1 in cancer therapy

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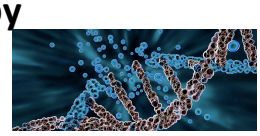

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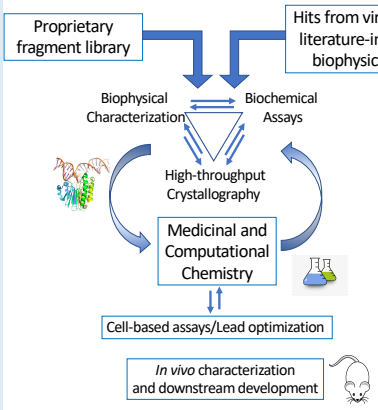
## Introduction

Cancer cells respond to increases in DNA damage by upregulating their DNA damage response (DDR). The base excision repair (BER) pathway corrects damage to single DNA bases through the action of multiple enzymes, including the central protagonist, apurinic/apyrimidinic endonuclease 1 (APE1). Numerous studies have shown association between increased APE1 levels and enhanced growth, migration, and drug resistance in human tumor cells, as well as with decreased patient survival. Inhibition of APE1 induces synthetic lethality (SL) in PTEN, BRCA, and ATM-deficient cells and has been implicated in over 20 human cancers, making APE1 an attractive target for developing anticancer therapies. Despite intensive effort, there are currently no clinical endonuclease inhibitors of APE1. We have used a newly developed high-throughput protein X-ray crystallography-based fragment screen to obtain starting points for the design of molecules to block APE1 function. Starting with a proprietary fragment library, we obtained high quality fragment-bound crystal structures showing diversity of chemical matter and hit location, representing the first experimental 3D structures of APE1 bound to drug-like molecules, thereby resolving a primary bottleneck in the path to inhibitor development. The implementation of this unique hit discovery campaign has facilitated three independent strategies toward the development of APE1 inhibitors, including (i) fragment growing and elaboration of hits bound at the endonuclease site; (ii) linking of fragments bound to distinct but proximally located sites, and (iii) use of fragments for the design of hooks to use in targeted protein degradation (TPD) strategies. We are using a combination of computational and medicinal chemistry, structural biology, and biochemical and biophysical studies and will discuss our progress towards these goals.

## DDR drug discovery platform at XPose Therapeutics



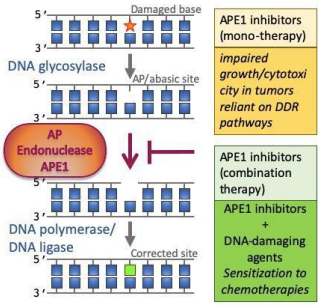

| Program / Stages | Target selection | Target production | Solved structures | Hit identification | Lead generation | Lead optimization | IND enabling |
|---|---|---|---|---|---|---|---|
| | | | | Discovery/IP generation | Discovery/IP generation | Discovery/IP generation | Preclinical |
| APE1 | | | | | | | |
| FEN1 | | | | | | | |
| ExoN | | | | | | | |
| UNG (UDG) | | | | | | | |
| WRN | | | | | | | |

XPose ("X-ray Pose") Therapeutics leverages 3D structures of molecular fragments bound to drug targets to develop specific therapeutics. Using an innovative drug discovery platform, XPose Therapeutics is pursuing targets in the DDR space traditionally considered challenging or 'undruggable'

## APE1 - a novel target in cancer

- Broad therapeutic utility: high expression in >20 cancers including prostate, lung, ovarian, cervical, pancreatic, colon, and leukemias
- Highly promising in glioblastoma (5-Yr survival ~5%) where elevated APE1 may cause resistance to the current standard of care temozolomide
- Combination therapies: inhibition of APE1 potentiates effects of cell damaging agents while overexpression renders cell lines resistant to chemotherapy
- Synthetic lethality: inhibition of APE1 induces synthetic lethality in PTEN, BRCA, and ATM-deficient cells
- Safety: APE1 knockdown has no effect in non-dividing peripheral neurons but growth inhibition in tumor cells
- First in class: Inhibitors of APE1 endonuclease activity have not advanced beyond the hit-to-lead stage despite HTS and *in silico* efforts

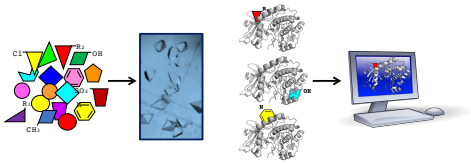
## Role of APE1 in Base Excision Repair (BER)




APE1 recognizes abasic sites and cleaves the adjacent DNA backbone to facilitate repair. APE1 is responsible for >95% of the AP endonuclease activity in the cell

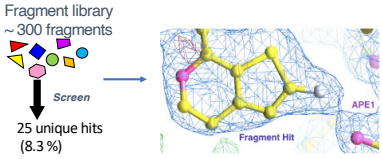
## High-throughput Crystallography for Hit Discovery

ACCELERO Biostructures



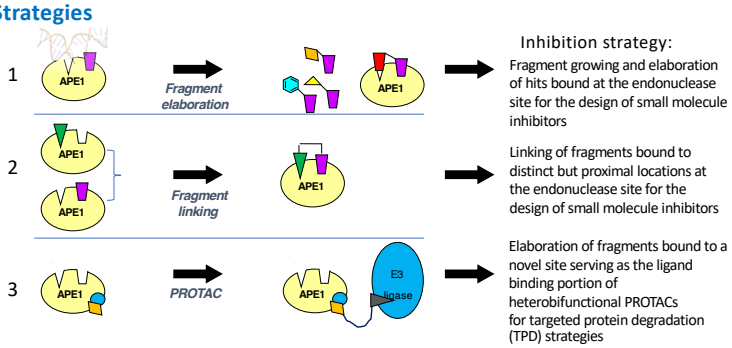

## Results of APE1 Crystallography-based Hit Discovery Campaign

We identified APE1 crystallization conditions compatible with fragment screening and developed a scalable system to obtain hundreds of crystals. We then screened a ~300 fragment library and obtained 25 high quality structures showing unique and diverse fragment hits (8.3% hit rate). Hits bound at the endonuclease site, but also at a previously unidentified secondary site.




Fragment- and structure-based drug discovery for developing therapeutic agents targeting the DNA Damage Response. Wilson DM 3rd, Deacon AM, Duncton MAJ, Pellicena P, Georgiadis MM, Yeh AP, Arvai AS, Moiani D, Tainer JA, Das D. Prog Biophys Mol Biol. 2020 Oct 25:S0079-6107(20)30111-5. doi: 10.1016/j.pbiomolbio.2020.10.005

## A Single Hit Discovery Campaign Leads to Multiple APE1 Inhibition Strategies




Inhibition strategy:

Fragment growing and elaboration of hits bound at the endonuclease site for the design of small molecule inhibitors

Linking of fragments bound to distinct but proximal locations at the endonuclease site for the design of small molecule inhibitors

Elaboration of fragments bound to a novel site serving as the ligand binding portion of heterobifunctional PROTACs for targeted protein degradation (TPD) strategies

## Conclusion

The DNA repair enzyme APE1 is a highly promising target for the development of anticancer therapies. Despite intensive effort, there are currently no clinical endonuclease inhibitors of APE1. XPose Therapeutics is employing a newly developed high-throughput protein X-ray crystallography-based fragment screen to obtain starting points for the design of drugs to block APE1 function. Based on this technology, a single hit discovery campaign has facilitated three independent strategies toward the development of APE1 inhibitor leads, including (i) fragment growing and elaboration of hits bound at the endonuclease site; (ii) linking of fragments bound to distinct but proximally located sites, and (iii) use of fragments for the design of ligands to use in targeted protein degradation (TPD) strategies. A combination of computational and medicinal chemistry, structural biology, and biochemical and biophysical studies will allow XPose Therapeutics to quickly drive the design and development of functional inhibitors APE1 and of other DDR targets in its pipeline.

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